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(54) Title: CYCLIC ANALOGS OF PTH AND PTHrP

(57) Abstract

Cyclic analogs of PTH and PTHrP wherein a disulfide or amide bond links the side chains of residues A_{13} and A_{17}, A_{26} and A_{30}, or A_{13} and A_{17} and A_{26} and A_{30}.

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CYCLIC ANALOGS OF PTH AND PTHrP

Background of the Invention

Parathyroid hormone ("PTH") is a polypeptide produced by the parathyroid glands. The mature circulating form of the hormone is comprised of 84 amino acid residues. Parathyroid hormone-related protein ("PTHrP") is a 139 to 173 amino acid-protein with N-terminal homology to PTH. PTHrP shares many of the biological effects of PTH including binding to a common PTH/PTHrP receptor. See Chipani, E., et al., Endocrinology, 1993 132, 2157-2165; Broadus, A.E., Steward, A.F., Parathyroid hormone-related protein: In: The Parathyroids, Bilezikian, J.P., et al., Eds, Raven Press, NY 1994, 259-294. Many homologs of both PTH and PTHrP have been characterized. See Nissenson, R., et al., Structure & Function of the Receptor for Parathyroid Hormone and Parathyroid Hormone-Related Protein, 3 Receptor 193-202, 1993; and Burtis, W.J., 38(11) Clinical Chemistry 2171-2183 (1992).

PTH has been shown to effect a positive bone balance. See Dempster, D.W., et al., Endocrine Rev., 1993, 14, 690-709; and Riggs, L., Amer. J. Med., 1991, 91 (Suppl 5B), 37S-41S. The anabolic effect of intermittently administered PTH has been observed in osteoporotic men (Slovik, D.M., et al., J. Bone Miner. Res., 1986, 1, 377-381), women (Reeve, J., et al., Br. Med. J., 1990, 301, 314-318), and with concurrent antiresorptive therapy (Hesch, R-D., et al., Calcif Tissue Int, 1989, 176-180).

Summary of the Invention

In one aspect, the invention relates to cyclic peptide analogs of PTH covered by the following generic formula:

$$R_1 \diagdown A_1\text{-Val-Ser-Glu-}A_5\text{-Gln-}A_7\text{-}A_8\text{-His-Asn-Leu-}A_{12}\text{-}A_{13}\text{-His-}$$
$$R_2 \diagup$$
$$A_{15}\text{-}A_{16}\text{-}A_{17}\text{-}A_{18}\text{-Glu-Arg-}A_{21}\text{-}A_{22}\text{-}A_{23}\text{-Leu-}A_{25}\text{-}A_{26}\text{-Lys-}$$
$$\text{Leu-Gln-}A_{30}\text{-Val-}A_{32}\text{-}A_{33}\text{-}A_{34}\text{-}R_3$$

wherein:

A_1 is Ser or Ala;

A_5 is Ile or Met;

A_7 is Leu or Phe;

A_8 is Met, Nle, or Val;

A_{12} is Gly, Glu, Aib, Ala, or D-Ala;

A_{13} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, -NHCH(CH_2NH_2)CO-, -NHCH((CH_2)$_2$$NH_2$)CO-, Asp, Glu, -NHCH(($CH_2$)$_3$COOH)CO-, and -NHCH(($CH_2$)$_4$COOH)CO-;

A_{15} is Leu, or Arg;

A_{16} is Ser, His, Asn, or Ala;

A_{17} is the D- or L- isomer selected from the group consisting of Ser, Thr, Cys, Hcy, Lys, Orn, -NHCH(CH_2NH_2)CO-, -NHCH((CH_2)$_2$$NH_2$)CO-, Asp, Glu, -NHCH(($CH_2$)$_3$COOH)CO-, and -NHCH(($CH_2$)$_4$COOH)CO-;

A_{18} is Met, Leu, Nle, or Val;

A_{21} is Met, Leu, Nle, Gln, or Val;

A_{22} is Glu, Asp, or Gln;

A_{23} is Trp, 1-Nal, or 2-Nal;

A_{25} is Arg, or Gln;

A_{26} is the D- or L- isomer selected from the group consisting of Met, Cys, Hcy, Lys, Orn, -NHCH(CH_2NH_2)CO-, -NHCH((CH_2)$_2$$NH_2$)CO-, Asp, Glu, -NHCH(($CH_2$)$_3$COOH)CO-, and -NHCH(($CH_2$)$_4$COOH)CO-;

A_{30} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, -NH-CH(CH_2NH_2)CO-, -NHCH((CH_2)$_2$$NH_2$)CO-, Asp, Glu, -NHCH(($CH_2$)$_3$COOH)CO-, and

$-NHCH((CH_2)_4COOH)CO-$;

A_{32} is His or is deleted;

A_{33} is Asn, Ser, or is deleted;

A_{34} is Ala, Phe, p-X-Phe (where X is a halogen, CH_3, or OH), or is deleted;

each of R_1 and R_2 is, independently, H, C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} napthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenyl, C_{11-20} hydroxynapthylalkyl, or COE_1 where E_1 is C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} napthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenylalkyl, or C_{11-20} hydroxynapthylalkyl;

R_3 is OH, NH_2, C_{1-12} alkoxy, or $NH-Y-CH_2-Z$ where Y is a C_{1-12} hydrocarbon moiety and Z is H, OH, CO_2H or $CONH_2$; or a pharmaceutically acceptable salt thereof; and

a disulfide or amide bond links the side chains of residues A_{13} and A_{17}, A_{26} and A_{30}, or A_{13} and A_{17} and A_{26} and A_{30}.

The following are examples of the cyclic peptides of this invention as covered by the above formula: c[Lys^{13}, Asp^{17}]hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]bPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}] rPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}][$Nle^{8,18}$, Tyr^{34}]hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}][$Nle^{8,18}$,Tyr^{34}]rPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}] [$Nle^{8,18}$, Tyr^{34}]bPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}]hPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}]bPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}]rPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]hPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}] [$Nle^{8,18}$, Tyr^{34}]bPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}] rPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]bPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]rPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}] hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]rPTH (1-34)NH_2; or c[Lys^{13}, Asp^{17}] c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]bPTH(1-34)NH_2; or a pharmaceutically acceptable salt thereof.

In another aspect, the invention relates to cyclic peptide analogs of PTHrP covered by the following generic formula:

$$\begin{array}{l} R^1 \\ \quad \backslash \\ \quad\ \text{Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-} \\ \quad / \\ R_2 \\ \quad A_{13}\text{-Ser-Ile-Gln-}A_{17}\text{-Leu-Arg-Arg-Arg-}A_{22}\text{-Phe-Leu-} \\ \quad A_{25}\text{-}A_{26}\text{-Leu-Ile-}A_{29}\text{-}A_{30}\text{-}A_{31}\text{-}A_{32}\text{-}A_{33}\text{-}A_{34}\text{-}R_3 \end{array}$$

wherein:

A_{13} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, $-NHCH(CH_2NH_2)CO-$, $-NHCH((CH_2)_2NH_2)CO-$, Asp, Glu, $-NHCH((CH_2)_3COOH)CO-$, and $-NHCH((CH_2)_4COOH)CO-$;

A_{17} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, $-NHCH(CH_2NH_2)CO-$, $-NHCH((CH_2)_2NH_2)CO-$, Asp, Glu, $-NHCH((CH_2)_3COOH)CO-$, and $-NHCH((CH_2)_4COOH)CO-$;

A_{22} is Phe or Ile;

A_{25} is His or Gln;

A_{26} is the D- or L- isomer selected from the group consisting of His, Asn, Cys, Hcy, Lys, Orn, $-NHCH(CH_2NH_2)CO-$, $-NHCH((CH_2)_2NH_2)CO-$, Asp, Glu, $-NHCH((CH_2)_3COOH)CO-$, and $-NHCH((CH_2)_4COOH)CO-$;

A_{29} is Ala or Glu;

A_{30} is the D- or L- isomer selected from the group consisting of Glu, Gly, Cys, Hcy, Lys, Orn, $-NHCH(CH_2NH_2)CO-$, $-NHCH((CH_2)_2NH_2)CO-$, Asp, Glu, $-NHCH((CH_2)_3COOH)CO-$, and $-NHCH((CH_2)_4COOH)CO-$;

A_{31} is Ile or Val; and

A_{32} is His, Asn, or is deleted;

A_{33} is Thr or is deleted;

A_{34} is Ala or is deleted;

each of R_1 and R_2 is, independently, H, C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} napthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenyl, C_{11-20}

hydroxynapthylalkyl, or COE_1 where E_1 is C_{1-12} alkyl, C_{7-20} phenylalkyl, C_{11-20} napthylalkyl, C_{1-12} hydroxyalkyl, C_{7-20} hydroxyphenylalkyl, or C_{11-20} hydroxynapthylalkyl;

R_3 is OH, NH_2, C_{1-12} alkoxy, or NH-Y-CH_2-Z where Y is a C_{1-12} hydrocarbon moiety and Z is H, OH, CO_2H or $CONH_2$; or a pharmaceutically acceptable salt thereof; and

a disulfide or amide bond links the side chains of residues A_{13} and A_{17}, A_{26} and A_{30}, or A_{13} and A_{17} and A_{26} and A_{30}.

The following are examples of the cyclic peptide of this invention as covered by the above formula: c[Lys^{13}, Asp^{17}]hPTHrP(1-34)NH_2; c[Lys^{26} , Asp^{30}]hPTHrP(1-34)NH_2; or c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]hPTHrP(1-34)NH_2; or a pharmaceutically acceptable salt thereof.

With the exception of the N-terminal amino acid, all abbreviations (e.g. Ala or A_1) of amino acids in this disclosure stand for the structure of -NH-CH(R)-CO-, wherein R is a side chain of an amino acid (e.g., CH_3 for Ala). For the N-terminal amino acid, the abbreviation stands for the structure of =N-CH(R)-CO-, wherein R is a side chain determinant of an amino acid. 1-Nal, 2-Nal, Nle, Orn, Hcy and Aib are respective abbreviations of the following α-amino acids: 3-(1-naphthyl)alanine, 3-(2-naphthyl)alanine, norleucine, ornithine, homocysteine and α-aminoisobutyric acid, respectively. Also, in the above formula, hydroxyalkyl, hydroxyacyl, hydroxyphenyl-alkyl, and hydroxynaphthyl alkyl may contain 1-4 hydroxy substituents, and COE_1, stands for -C=O•E_1 Examples of -C=O•E_1 include, but are not limited to, acetyl and phenylpropionyl.

In this disclosure, the disulfide or amide bond which links two residues in a peptide of this invention are formed between the side-chain functionalities. This is, between the side-chain carboxyl group of an acidic amino acid residue (e.g., Asp, Glu, -NH((CH_2)$_3$COOH)CO-, or

-NH((CH$_2$)$_4$COOH)CO-) and the side-chain amino group of a basic amino acid residue (e.g., Lys, Orn, -NHCH(CH$_2$NH$_2$)CO-, or -NHCH(CH$_2$)$_2$NH$_2$)CO-), or between the side-chain sulfhydryl groups of two Cys residues. In both formulas set forth herein, the amide or disulfide bond between two residues is not shown. A peptide of this invention is also denoted herein by another format, e.g., c[Lys^{13},Asp^{17}][Nle^{8,18},Tyr^{34}]bPTH(1-34)NH$_2$, with the two linked residues placed between two brackets following "c" (e.g., Lys^{13} and Asp^{17}), with substituted amino acids from the natural sequence placed between the second set of brackets (e.g., Nle^{8} for Met^{28}, Nle^{18} for Met^{18}, and Tyr^{34} for Phe^{34} in bPTH). The abbreviation bPTH stands for bovine PTH, rPTH for rat PTH, hPTH for human PTH, and hPTHrP for human PTHrP. The numbers between the parenthesis refer to the number of amino acids present in the peptide (e.g., the first 34 amino acids of bPTH).

In another embodiment, the side-chain functionalities of amino acid residues A$_{13}$ and A$_{17}$, A$_{26}$ and A$_{30}$, or A$_{13}$ and A$_{17}$ and A$_{26}$ and A$_{30}$ constitute a lanthionine bridge. Examples of lanthionine side-chain bridges are thioethers (e.g., -(CH$_2$)$_n$-S-(CH$_2$)$_m$- where m and n, independently, are 1-3) or dithioethers (e.g., -(CH$_2$)$_m$-S-(CH$_2$)$_n$-S-(CH$_2$)$_o$- where m, n, and o, independently, are 1-3. Examples of the synthesis of peptides containing lanthionines is described in Fukase, K., et al., Tetrahedron Let. 29:795-798 (1988); Labl, M., et al., Tetrahedron Let. 25:2067-2068 (1984); and Mosberg, H.I., Life Science 43:1013-1020 (1988).

The cyclic peptides of the invention can be used to stimulate the growth of bone in a subject (a mammal such as a human subject). Thus, the cyclic peptide are useful in the treatment of osteoporosis and bone fractures. The cyclic peptides of the invention can be

administered concurrently with antiresorptive therapy, e.g., bisphosphonate and calcitonin.

The cyclic peptides of this invention can be provided in the form of pharmaceutically acceptable salts. Examples of such salts include, but are not limited to, those formed with organic acids (e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, methanesulfonic, toluenesulfonic, or pamoic acid), inorganic acids (e.g., hydrochloric acid, sulfuric acid, or phosphoric acid), polymeric acids (e.g., tannic acid, carboxymethyl cellulose, polylactic, polyglycolic, or copolymers of polylactic-glycolic acids).

A therapeutically effective amount of a cyclic peptide of this invention and a pharmaceutically acceptable carrier substance (e.g., magnesium carbonate, lactose, or a phospholipid with which the therapeutic compound can form a micelle) together form a therapeutic composition (e.g., a pill, tablet, capsule, or liquid) for administration (e.g., orally, intravenously, transdermally, pulmonarily, vaginally, subcutaneously, nasally, iontophoretically, or by intratracheally) to a subject in need of the peptide. The pill, tablet, or capsule can be coated with a substance capable of protecting the composition from the gastric acid or intestinal enzymes in the subject's stomach for a period of time sufficient to allow the composition to pass undigested into the subject's small intestine. The therapeutic composition can also be in the form of a biodegradable or nonbiodegradable sustained release formulation for subcutaneous or intramuscular administration. See, e.g., U.S. Patents 3,773,919 and 4,767,628 and PCT Application No. WO 94/00148. Continuous administration can also be obtained using an implantable or external pump (e.g., INFUSAID™ pump) to

administer the therapeutic composition. The cyclic peptide can be administered intermittently, e.g., single daily injection, or continuously at a low dose, e.g., sustained release formulation.

The dose of a cyclic peptide of the present invention for treating the above-mentioned diseases or disorders varies depending upon the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such an amount of the cyclic peptide as determined by the attending physician or veterinarian is referred to herein as a "therapeutically effective amount."

Also contemplated within the scope of this invention is a cyclic peptide covered by the above generic formulas for use in treating diseases or disorders associated with the need to stimulate bone growth, e.g., osteoporosis or fractures.

Other features and advantages of the present invention will be apparent from the detailed description and from the claims.

Description of the Preferred Embodiments

Synthesis

The peptides of the invention can be prepared by standard solid phase synthesis. See, e.g., Stewart, J.M., et al., Solid Phase Synthesis (Pierce Chemical Co., 2d ed. 1984). The following is a description of how Analog #1 was prepared. Other peptides of the invention can be prepared in an analogous manner by a person of ordinary skill in the art.

Analog I was synthesized on an APPLIED BIOSYSTEMS™ 430A Automated Peptide Synthesizer (Applied Biosystems Inc., Foster City, CA) using version 1.40 of the software

for NMP/HOBt Boc based chemistry. The following side-chain protected amino acid derivatives obtained from Applied Biosystems, Inc. were used in the course of the synthesis: N-Boc-Arg(N^G-Tosyl)-OH, N-Boc-Asp(cHex)-OH, N-Boc-Glu(OBzl)-OH, N-Boc-His(Bom)-OH, N-Boc-(2-Cl-Z)-OH, N-Boc-Ser(Bzl)-OH, N-Boc-Thr(Bzl)-OH. N-Boc-Asp(OFm)-OH and N-Boc-Lys(Fmoc)-OH were purchased from Bachem, CA (Torrance, CA). The synthesis was carried out a *p*-methylbenzhydrylamine HCl resin (0.57 meq N/g)(Applied Biosystems, Inc.) at a 0.5 mmol scale until residue Arg^{21} when the synthesis was split and carried out at a 0.25 mmol scale until completion. All three Arg residues at positions 19-21 were double-coupled and then capped with Ac_2O.

The first four residues were coupled using the above automated synthesis. Extension of the fully protected resin-bound peptide N-Boc-Ile-His(Bom)-Thr(Bzl)-Ala-O-Resin was then carried out manually on a A5-6023 Variable-Rate Flask Shaker (St. John Assoc. Inc., Beltsville, MD). Amino acid residues at positions 26-30 were manually incorporated, and the lactam ring was formed before reconvening the automated solid phase peptide synthesis. Each manual cycle included the following steps: 1) Dimethylchloride (DCM) wash (3 x 1 min); 2) Tetrahydorfuric acid (TFA) 50% in DCM (1 x 3 min, 1 x 20 min); 3) DCM wash (3 x 1 min); 4) Diisopropylethylamine (DIEA) 1.5% in DCM (2 x 1 min); 5) DIEA 1.5% in NMP (2 x 1 min); 6) DCM wash (3 x 1 min); 7) NMP wash (3 x 1 min); and 8) coupling: 2 mmol (4eq.) of Boc-amino acid + 2 mmol of HOBt in NMP + 2 mmol of (diisopropylcarbodiimide) DIC and up to 13 ml of total volume with NMP. After 1 hour, 2 ml of dimethylsulfoxide (DMSO) were added. The reaction was checked with ninhydrin test. [Reaction times: Asp(OFm) 1.5 hrs.; Ala: 1.5 hrs.; Ile: 1.5 hrs.; Leu: 1.5 hrs.; Lys(FMOC): 2.5

hrs.; 9) NMP wash (3 x 1 min); and 10) DCM wash (3 x 1 min).

The cyclization was accomplished by coupling side chains in the following manner: 1) Deprotection with pipedrine 20% in NMP (1 x 3', 1 x 20 min); 2) DCM wash (3 x 1 min); 3) NMP wash (3 x 1 min); 4) Coupling with benzotriazolyl-N-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) 1.5 mmol (3 eq.) in dimethylformamide (DMF) (1 ml) + 1.5% DIEA in NMP (12 ml) for 3 hours (negative ninhydrin test); 5) NMP wash (3 x 1 min); 6) DCM wash (3 x 1 min); 7) acetic anhydride 5% in NMP (1 x 10 min); 8) NMP wash (3 x 1 min); and 9) DCM wash (3 x 1 min).

The remaining 25 residues were coupled using the automated synthesis described above. The final side-chain protected peptidyl-resin (1.8 g.) was cleaved with HF/anisole (10% at -5°C for 75 min). After removal of the HF under reduced pressure, the residue was washed consecutively with hexane and diethyl ether and filtered. The crude peptide was separated from the resin using 50% aqueous AcOH and the solution was lyophilized. The analytical HPLC profile of the crude peptide show a major peak (t_R = 23.20 min.) corresponding to the product.

The crude peptide was purified with preparation HPLC on a VYDAC® protein C-18 reverse-phase column (5 x 30 cm) (Waters, Milford, MA) using the following solvent system: A = 0.1% TFA in water and B = 0.1% TFA in acetonitrile. The linear gradient used was: 0 - 10 min (0 - 10% B); and 10 - 200 min (10 - 50% B). The flow rate was 70 ml/min and fractions of 20 ml were collected and analyzed on an analytical HPLC. The pure fractions were pooled and lyophilized.

The full names for the abbreviations used above are as follows: Boc for 1-butyloxycarbonyl, OFm for O-formyl, OBzl is O-benzyl, BOM for benzyloxymethyl, Bzl

for benzyl, N^G-Tosyl for tosyl at guanidyl site, HOBt for 1-hydroxybenzotriazole, NMP for N-methyl-2-pyrrolidone, Fmoc for 9-Fluoronylmethyloxycarbonyl, 2-Cl-Z for 2-chlorobenzyloxycarbonyl and O-cHex for O-cyclohexyl.

Other cyclic lactams of this invention can be prepared in an analogous manner by a person of ordinary skill in the art. Moreover, the disulfide bridge formation between the two Cys residues of a cyclic peptide of this invention can be achieved following general procedures described in the prior art. For example, see Coy, et al., U.S. Patent No. 4,853,371; M. Bodanszky, et al., Chapter 6, Vol. 21, Chapter 6, Vol. 16, The Practice of Peptide Synthesis (Springer-Verlag, 1984).

PTH Receptor Binding

The cyclic peptide of the invention can be tested for the ability to bind to the PTH receptor present on SaOS-2 (human osteosarcoma cells). Saos-2 cells (American Type Culture Collection, Rockville, MD; ATCC #HTB 85) are maintained in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) and 2 mM glutamine at 37°C in a humidified atmosphere of 5% CO_2 in air. The medium is changed every three or four days, and the cells are subcultured every week by trypsinization.

Saos-2 cells are maintained for four days after they have reached confluence. The medium is replaced with 5% FBS in RPMI 1640 medium and incubated for 2 hrs at room temperature with 10 x 10^4cpm mono-^{125}I-[Nle^{8,18}, Tyr^{34}(3-^{125}I)]bPTH(1-34)NH_2 in the presence of a competing cyclic peptides of the invention, at various concentrations between 10^{-11}M to 10^{-4} M. The cells are washed four times with ice-cold PBS and lysed with 0.1 M NaOH, and the radioactivity associated with the cells is

counted in a scintillation counter. Synthesis of mono-^{125}I-[Nle^{8,18}, Tyr^{34}(3-^{125}I)] bPTH(1-34)NH_2 is carried out as described in Goldman, M.E., et al., Endocrinology, 1988, 123, 1468-1475.

The binding assay was conducted with Analog I. The IC_{50} (half maximal inhibition of binding of mono-^{125}I-[Nle^{8,18}, Tyr^{34}(3-^{125}I)]bPTH(1-34)NH_2) for Analog I was calculated to be 500 nM.

Stimulation of Adenylate Cyclase Release

The ability of the cyclic analogs of the invention to induce a biological response in SaOS-2 cells can also be measured. For example, the stimulation of the adenylate cyclase can be determined by measuring the level of synthesis of cAMP(adenosine 3':5'-cyclic monophosphate) as described previously in Rodan, et al., 1983, J. Clin. Invest. 72, 1511 and Goldman, et al., 1988, Endocrinology, 123, 1468. Confluent SaOS-2 cells in 24 wells plates are incubated with 0.5 μCi[^{3}H]adenine (26.9 Ci/mmol, New England Nuclear, Boston, MA) in fresh medium at 37°C for 2 hrs, and washed twice with Hank's balanced salt solution (Gibco, Gaithersburg, MD). The cells are treated with 1 mM IBMX [isobutylmethylxanthine, Sigma, St. Louis, MO] in fresh medium for 15 min, and the cyclic peptides are added to the medium to incubate for 5 min. The reaction is stopped by the addition of 1.2 M trichloroacetic acid (TCA) (Sigma, St. Louis, MO) followed by sample neutralization with 4 N KOH. cAMP is isolated by the two-column chromatographic method (Salmon, et al., 1974, Anal. Biochem. 58, 541). The radioactivity is counted in a scintillation counter (Liquid Scintillation Counter 2200CA, PACKARD, Downers Grove, IL).

The EC_{50}'s (half maximal stimulation of adenylate cyclase) for Analog I was calculated to be 20 nM. The

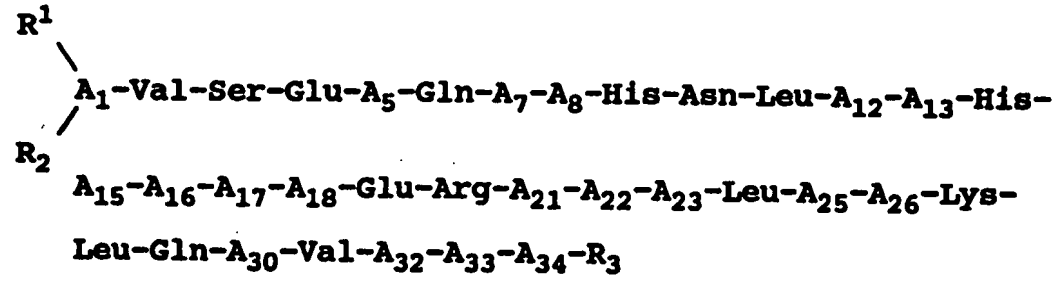
cyclic peptide, thus, was a potent stimulator of adenylate cyclase activity in SaOS-2 cells. This biochemical pathway has been indicative as a proximal signal for osteoblast proliferation (e.g., bone growth).

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the claims.

What is claimed is:

1. A cyclic polypeptide of the formula:



wherein:

A_1 is Ser or Ala;

A_5 is Ile or Met;

A_7 is Leu or Phe;

A_8 is Met, Nle, or Val;

A_{12} is Gly, Glu, Aib, Ala, or D-Ala;

A_{13} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, $NHCH(CH_2NH_2)CO$, $NHCH((CH_2)_2NH_2)CO$, Asp, Glu, $NHCH((CH_2)_3COOH)CO$, and $NHCH((CH_2)_4COOH)CO$;

A_{15} is Leu, or Arg;

A_{16} is Ser, His, Asn, or Ala;

A_{17} is the D- or L- isomer selected from the group consisting of Ser, Thr, Cys, Hcy, Lys, Orn, $NHCH(CH_2NH_2)CO$, $NHCH((CH_2)_2NH_2)CO$, Asp, Glu, $NHCH((CH_2)_3COOH)CO$, and $NHCH((CH_2)_4COOH)CO$;

A_{18} is Met, Leu, Nle, or Val;

A_{21} is Met, Nle, Gln, or Val;

A_{22} is Glu, Asp, or Gln;

A_{23} is Trp, 1-Nal, or 2-Nal;

A_{25} is Arg, or Gln;

A_{26} is the D- or L- isomer selected from the group consisting of Met, Cys, Hcy, Lys, Orn, $NHCH(CH_2NH_2)CO$, $NHCH((CH_2)_2NH_2)CO$, Asp, Glu, $NHCH((CH_2)_3COOH)CO$, and $NHCH((CH_2)_4COOH)CO$;

A_{30} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, NH-CH(CH_2NH_2)CO, NHCH((CH_2)$_2NH_2$)CO, Asp, Glu, NHCH((CH_2)$_3$COOH)CO, and NHCH((CH_2)$_4$COOH)CO;

A_{32} is His or is deleted;

A_{33} is Asn, Ser, or is deleted;

A_{34} is Ala, Phe, p-X-Phe (where X is a halogen, CH_3, or OH), or is deleted;

each R_1 and R_2 is, independently, H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkynyl, phenyl, napthyl, C_{7-10} phenylalkyl, or C_{1-12} acyl;

R_3 is OH, C_{1-12} alkoxy, C_{7-10} phenylalkoxy, C_{8-20} napthylalkoxy, or NR_1R_2; and

a disulfide or amide bond links the side chains of residues A_{13} and A_{17}, A_{26} and A_{30}, or A_{13} and A_{17} and A_{26} and A_{30}.

2. A cyclic polypeptide of claim 1, wherein A_5 is Ile; A_7 is Phe or Leu; A_8 is Met or Nle; A_{12} is Gly; A_{15} is Leu; A_{16} is Ser, Asn, or Ala; A_{18} is Met, Val, or Nle; A_{21} is Met or Val; A_{22} is Glu or Gln; A_{23} is Trp; A_{25} is Arg; A_{32} is His; A_{33} is Asn; and
A_{34} is Phe or Try.

3. A cyclic polypeptide of claim 2, wherein A_{13} is Lys; A_{17} is Asp; A_{26} is Lys; A_{30} is Asp; and an amide bond links the side chains of A_{13} and A_{17}; or a pharmaceutically acceptable salt thereof.

4. A cyclic polypeptide of claim 3, wherein said cyclic polypeptide is c[Lys^{13}, Asp^{17}]hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]bPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]rPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}][$Nle^{8,18}$, Tyr^{34}]hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}][$Nle^{8,18}$,Tyr^{34}]rPTH(1-34)NH_2; or c[Lys^{13},

Asp^{17}][$Nle^{8,18}$, Tyr^{34}]bPTH(1-34)NH_2; or a pharmaceutically acceptable salt thereof.

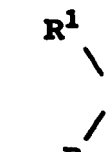
5. A cyclic polypeptide of claim 3, wherein A_{13} is Lys; A_{17} is Ser; A_{26} is Lys; A_{30} is Asp; and an amide bond links the side chains of A_{26} and A_{30}; or a pharmaceutically acceptable salt thereof.

6. A cyclic polypeptide of claim 5, wherein said cyclic polypeptide is c[Lys^{26}, Asp^{30}]hPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}]bPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}]rPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]hPTH(1-34)NH_2; c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]bPTH(1-34)NH_2; or c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]rPTH(1-34)NH_2; or a pharmaceutically acceptable salt thereof.

7. A cyclic polypeptide of claim 2, wherein A_{13} is Lys; A_{17} is Asp; A_{26} is Lys; A_{30} is Asp; and a first amide bond links the side chains of A_{13} and A_{17} and a second amide bond links the side chains of A_{26} and A_{30}; or a pharmaceutically acceptable salt thereof.

8. A cyclic polypeptide of claim 3, wherein said cyclic polypeptide is c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]bPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]rPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}] hPTH(1-34)NH_2; c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]rPTH (1-34)NH_2; or c[Lys^{13}, Asp^{17}] c[Lys^{26}, Asp^{30}][$Nle^{8,18}$, Tyr^{34}]bPTH(1-34)NH_2; or a pharmaceutically acceptable salt thereof.

9. A cyclic polypeptide of the formula:



Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-

A_{13}-Ser-Ile-Gln-A_{17}-Leu-Arg-Arg-Arg-A_{22}-Phe-Leu-A_{25}-

A_{26}-Leu-Ile-A_{29}-A_{30}-A_{31}-A_{32}-A_{33}-A_{34}-R_3

wherein:

A_{13} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, $NHCH(CH_2NH_2)CO$, $NHCH((CH_2)_2NH_2)CO$, Asp, Glu, $NHCH((CH_2)_3COOH)CO$, and $NHCH((CH_2)_4COOH)CO$;

A_{17} is the D- or L- isomer selected from the group consisting of Cys, Hcy, Lys, Orn, $NHCH(CH_2NH_2)CO$, $NHCH((CH_2)_2NH_2)CO$, Asp, Glu, $NHCH((CH_2)_3COOH)CO$, and $NHCH((CH_2)_4COOH)CO$;

A_{22} is Phe or Ile;

A_{25} is His or Gln;

A_{26} is the D- or L- isomer selected from the group consisting of His, Asn, Cys, Hcy, Lys, Orn, $NHCH(CH_2NH_2)CO$, $NHCH((CH_2)_2NH_2)CO$, Asp, Glu, $NHCH((CH_2)_3COOH)CO$, and $NHCH((CH_2)_4COOH)CO$;

A_{29} is Ala or Glu;

A_{30} is the D- or L- isomer selected from the group consisting of Glu, Gly, Cys, Hcy, Lys, Orn, $NHCH(CH_2NH_2)CO$, $NHCH((CH_2)_2NH_2)CO$, Asp, Glu, $NHCH((CH_2)_3COOH)CO$, and $NHCH((CH_2)_4COOH)CO$, $[-(CH_2)_n-S-(CH_2)_m-]$;

A_{31} is Ile or Val; and

A_{32} is His, Asn, or is deleted;

A_{33} is Thr or is deleted;

A_{34} is Ala or is deleted;

each R_1 and R_2 is, independently, H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 where E_1 is C_{1-20} alkyl, C_{3-20}

alkenyl, C_{3-20} alkynyl, phenyl, napthyl, C_{7-10} phenylalkyl, or C_{1-12} acyl;

R_3 is OH, C_{1-12} alkoxy, C_{7-10} phenylalkoxy, C_{8-20} napthylalkoxy, or NR_1R_2; and

a disulfide or amide bond links the side chains of residues A_{13} and A_{17}, A_{26} and A_{30}, or A_{13} and A_{17} and A_{26} and A_{30}.

10. A cyclic polypeptide of claim 9, wherein A_{22} is Phe; A_{25} is His; A_{29} is Ala; A_{31} is Ile; A_{32} is His; A_{33} is Thr; and
A_{34} is Ala.

11. A cyclic polypeptide of claim 10, wherein A_{13} is Lys; A_{17} is Asp; A_{26} is His; A_{30} is Glu; and an amide bond links the side chains of A_{13} and A_{17}; or a pharmaceutically acceptable salt thereof.

12. A cyclic polypeptide of claim 11, wherein said cyclic polypeptide is c[Lys^{13}, Asp^{17}]hPTHrP(1-34)NH_2 or a pharmaceutically acceptable salt thereof.

13. A cyclic polypeptide of claim 10, wherein A_{13} is Lys; A_{17} is Asp; A_{26} is Lys; A_{30} is Glu; and an amide bond links the side chains of A_{26} and A_{30}; or a pharmaceutically acceptable salt thereof.

14. A cyclic polypeptide of claim 13, wherein said cyclic polypeptide agonist is c[Lys^{26}, Asp^{30}]hPTHrP(1-34)NH_2 or a pharmaceutically acceptable salt thereof.

15. A cyclic polypeptide of claim 10, wherein A_{13} is Lys; A_{14} is Asp; A_{26} is Lys; A_{30} is Glu; and an amide

bond links the side chains of A_{13} and A_{17} and A_{26} and A_{30}; or a pharmaceutically acceptable salt thereof.

16. A cyclic polypeptide of claim 15, wherein said cyclic polypeptide is c[Lys^{13}, Asp^{17}]c[Lys^{26}, Asp^{30}]hPTHrP(1-34)NH_2 or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09674

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 38/02; C07K 5/00, 7/00, 17/00
US CL :530/300, 317; 514/9

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 317; 514/9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Bone and Mineral Research, Volume 1, No. 4, issued 1986, D.M. Slovik et al, "Restoration of Spinal Bone in Osteoporotic Men by Treatment With Human Parathyroid Hormone (1-34) and 1,25-Dihydroxyvitamin D", pages 377-381, see entire document.	1-16
Y	Calcified Tissue International, Volume 44, issued 1989, R. D. Hesch et al, "Increase of Vertebral Density by Combination Therapy with Pulsatile 1-38hPTH and Sequential Addition of Calcitonin Nasal Spray in Osteoporotic Patients", pages 176-180, see entire document.	1-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 AUGUST 1996	Date of mailing of the international search report 05 SEP 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer AVIS M. DAVENPORT Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09674

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Endocrine Reviews, Volume 14, No. 6, issued December 1993, D.W. Dempster et al, "Anabolic Actions of Parathyroid Hormone on Bone", pages 690-709, see entire document.	1-16